STUDY OF ITRACONAZOLE

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by

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(2k22/MSCCHE/49)

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CANDIDATE'S DECLARATION

I, MANSI (2k22/MSCCHE/49) hereby certify that the work which is being presented in the dissertation entitled STUDY OF ITRACONAZOLE in partial fulfillment of the requirements for the award of the Degree of Masters of Science in Chemistry, submitted in the Department of Applied Chemistry, Delhi Technological University is an authentic record of my own work carried out during the period under the supervision of Prof. Ram Singh.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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CERTIFICATE

Certified that **MANSI (**2k22/MSCCHE/49) has carried out their research work presented in this dissertation entitled **"STUDY OF ITRACONAZOLE"** for the award of **Master of Science in Chemistry** from Department of Applied chemistry, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the dissertation do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

Prof. Ram Singh
Professor
Department of Applied chemistry
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ABSTRACT

This dissertation presents a comprehensive study of the Itraconazole, an FDA and CDSCO-approved medication primarily indicated for fungal infections. Itraconazole's efficacy stems from its binding affinity to plasma proteins within the human body, resulting in potent inhibition of cytochrome P450 and its associated enzymes, thereby augmenting its antifungal properties. The pharmacological mechanism of Itraconazole has been extensively investigated both in vitro and in vivo to elucidate its multifaceted actions. Beyond its established antifungal role, Itraconazole has garnered attention for its successful repurposing in treating viral infections, notably dengue, chikungunya, Ebola, and influenza. Emerging research has unveiled Itraconazole's potential in impeding cancer cell proliferation through modulation of various pathways including mTOR, VEGFR2, and Hh pathways. Notably, initiation of Itraconazole treatment curtailed up regulation of the Hh pathway, facilitating normal cellular processes of autophagy and apoptosis. This repurposing has shown promising outcomes in diverse cancers such as ovarian, breast, colorectal, liver, and basal cell carcinomas. Notably, in the context of the COVID-19 pandemic, Itraconazole has demonstrated potential in targeting the SARS-CoV-2 virus, specifically in its pre- and post-fusion conformations, exhibiting notable resistance against both MERS-CoV-2 and SARS-CoV-2 strains. The repurposing of Itraconazole represents a significant advancement in pharmacotherapy, extending its utility beyond fungal infections to encompass a spectrum of viral infections, cancer treatment, and potential mitigation against emerging infectious diseases such as COVID-19.

ACKNOWLEDGMENTS

I wish to express my sincerest gratitude to Prof. Ram Singh for his continuous guidance and mentorship that he provided me during the project. He showed me the path to achieve our targets by explaining all the tasks to be done and explained us the importance of this project as well as its industrial relevance. He was always ready to help and clear doubts regarding any hurdles in this project. Without his constant support and motivation, this dissertation would not have been successful.

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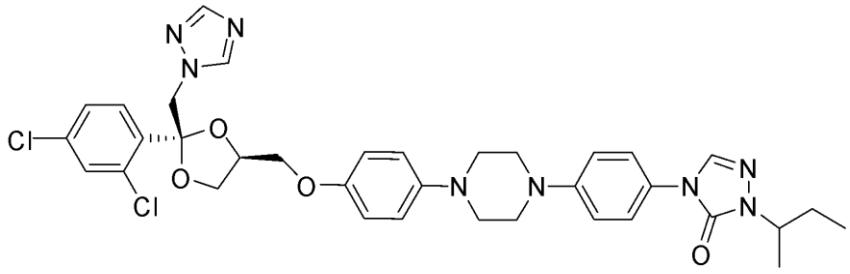
Place: Delhi MANSI

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

The antifungal drug Itraconazole was synthesized in 1978. The FDA has approved it in the USA and the national drug regulatory authority of India (Central Drugs Standard Control Organization). It is a triazole compound that has now developed into a broad-spectrum antifungal medicine. It has been administered to patients for the past 30 years in both oral (capsule and solution) and intravenous forms. In India, only 100mg oral capsule form is approved by the CDSCO. In 1984 when JAN VAN CUTSEM, carried out multiple successful in vitro analysis to understand the antifungal activity against *A. fumigatus*, *A. flavus*, and *A. nidulans* with much more better results in the BHI broth. There was a dependence of temperature and time in the cultures. Inhibition shown by Itraconazole was much more pronounced when the cultures were kept in a temperature of 37°C. A hyphal growth was observed in unreplenished cultures when compared to the replenished drug which might be due to the enervation of active bio-available drug present. But then again, the inhibition effects in the replenished was more in comparison to unreplenished drug. Similarly, in vivo studies were carried out where the animals were treated with polyethylene glycol 200 solvent and Itraconazole. Treatment done by the solvent lead to all its subject animal's death. But with treatment with Itraconazole majority of the animals showed progress and a few of them recovered completely. At the end of the trial, it was concluded that a maximum number of animals were free from the fungal infection and thus showing good inhibition powers presented by Itraconazole [Van Cutsem et al. 1984].

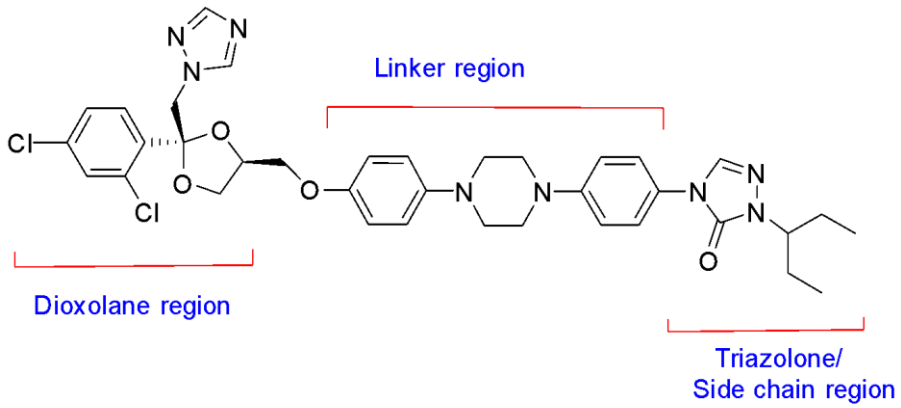


Itraconazole

2-Butan-2-yl-4-[4-[4-[4-[[(2R,4S)-2-(2,4-dichlorophenyl)-2-(1,2,4-triazol-1-ylmethyl) -1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-1,2,4-triazol-3-one

Structure and its chemistry

It belongs to a class of organic compounds called phenylpiperazines. Piperazine is a six-membered ring containing nitrogen atoms attached in opposite positions to each other [Almaghrabi 2021]. There are in total of three chiral carbons present in the itraconazole molecule. The two dioxolane rings each containing a chiral center, the triazolomethylene, and aryl oxymethylene are present in the *cis* position to each other. The clinical formulation is a 1:1:1:1 mixture of four stereoisomers (two enantiomeric pairs)



Itraconazole- Structure Activity Relationship

Indication

Itraconazole tends to behave as a broad-spectrum drug that is used for the treatment of fungal infections. It acts against all the pathogenic fungi except for the Zygomycetes. Due to the Itraconazole only being soluble in extremely acidic conditions, only an oral dosage formulation is used. Itraconazole tends to be released from the body almost immediately after the discontinuation of therapy i.e. there are no trace amounts found in the body apart from the nails and the skin, where the itraconazole persists for a longer period [Negroni and Arechavala 1993].

Itraconazole is used to treat many different types of fungal infections. A good deal of these fungal infections is rare but they can be unfavorable to the immunocompromised. The FDA approved itraconazole to be allowed as a remedy for blastomycosis, histoplasmosis, and aspergillosis. On the other hand, the FDA

has still not approved the use of this drug as a remedy for paracoccidioidomycosis, coccidioidomycosis, and candidiasis [Piérard et al 2000; Willems et al 2001]. Itraconazole is also used for the treatment of systemic fungal infections, where it acts as a prophylaxis. A large amount of the patients who use itraconazole as a prophylaxis are those who are being treated for HIV, undergoing chemotherapy, and those who have had organ transplants. The wide-spectrum coverage, safety profile, and minimal fungal resistance of Itraconazole help in offering excellent prophylaxis for immunocompromised patients [Westerberg and Voyack 2013].

There has been a recent development in which two new formulations of itraconazole are formed, an oral solution and an intravenous formulation, here the lipophilic Itraconazole combines with cyclodextrin. The improvement of solubility in Itraconazole has led to enhanced adsorption and bioavailability when compared with the original capsule formulation. This does not affect the tolerability profile of Itraconazole in any way. The additional adaptability property of Itraconazole offered by multiple passages of administration gives a little bit more room to treat patients of all kinds including children and those requiring intensive care [Willems et al 2001].

Absorption

Itraconazole pellet capsules weigh around 100 mg. Since it is a weak base, gas acidity is required for it to have a lower pH value to get ionized. It is advised to take the medicine after a meal for better absorption. In cases of low HCl in the stomach, the absorption of the drug will be decreased considerably. Hence acidic beverages increase the absorption of the drug while the antacids may hinder it [Heykants et al 1989; Cauwenbergh and Stoffels 1991]. Itraconazole tends to be soluble in lipids or fats. Patients who were administered in capsule form while fasting had considerably low plasma concentrations and these further led to treatment failures. The mean approximate peak for fasting patients was only 0.02 mg/L whereas those who were given the medicine after their meal had a much higher plasma concentration, while the mean approximate peak for it was 0.18 mg/L. The patients with higher plasma concentrations also showed better results in the drug treatment [Wishart 1987]

Distribution

Itraconazole is extremely protein-bound. *In vitro,* the activity of the drug in blood was found to be 99.8% bound, 94.9% showed it connected with plasma proteins, 4.9% bound with the blood cells, and the remaining 0.2% a free drug. The concentration of the drug in the kidney, liver, bone, stomach, spleen, and muscle is still relatively large regardless of its binding with the plasma protein [Grant and Clissold 1989]. In the structure of itraconazole, it is found that it has three nitrogen atoms in its azole ring just like the rest of the triazoles which might help in tissue penetration, extend the half-life, and increase the specificity of fungal enzymes [Alcántara and Garibay 1988]. In organic solvents such as dimethyl sulphoxide a concentration greater than 10 mg/mL had been achieved. Also, further addition of 5% dimethyl-β-cyclodextrin in the acidified polyethylene glycols and aqueous solutions led to a concentration of 5 mg/mL. The drug has little to no effect on mammalian cytochrome P450 enzymes at higher concentrations [Vanden Bossche et al 1986]. A study later itraconazole with interaction with animals suggests that it has a high-volume distribution and more half-life than other imidazoles. On repeated doses, it was found that the plasma concentrations were lower than the stable tissue concentration of itraconazole. The ratio of tissue to plasma level for the lung is ~3:1, for the liver, it's ~10:1, and for fat, it is ~25:1. This proved the capability of itraconazole for tissue penetration and is hence present continuously at the site of tissue infection [Van Cauteren et al 1987]. Aqueous body solutions such as tears, saliva, and CSF show little presence of the drug though body fluid with sufficiently abundant organic matter like sputum, bronchial exudates, and pus, tends to show a higher concentration [Prentice and Glasmacher 2005].

Pharmacokinetics

The various aspects of the pharmacodynamics and pharmacokinetics of Itraconazole can be replicated regardless of its formulation. Itraconazole is highly bound to plasma proteins and blood cells, forming a bond with these substances. As a result, its concentrations in body fluids, such as the eye and cerebrospinal fluid, are generally low. Even though it has a high affinity for plasma proteins, the concentrations of itraconazole in tissues are quite large, with an apparent volume of

around 11 liters per kg. Because itraconazole is a lipophilic antifungal agent, it is tissue-bound or protein concentration can be regarded as more relevant than its free counterpart [Heykants et al 1989].

In areas that are prone to fungal infections such as the skin, nails, and female genital tract, Itraconazole is found to accumulate in their tissues. The protein binding of Itraconazole is highly effective ensuring that the concentration of the drug at the site of infection remains higher than the relative plasma levels. After several days an equilibrium is established between the tissue and plasma furthermore, Itraconazole is excreted out from the infectious tissues. Therefore, Itraconazole was found to be very useful for the treatment of acute vaginal candidosis [Larosa et al 1986].

After the start of therapy, both the matrix and the bed of the nail show some signs of the presence of the drug [Matthieu et al 1991]. The itraconazole drug present in the infectious region of the nail is not distributed back to the plasma and gradually sheds normal growth. Hence, the antifungal therapy does not have to continue regularly. This allowed itraconazole pulse therapy for patients being treated for onychomycosis, where the drug is administered in a cynical regimen for one week out of every four [De Doncker et al 1997]. These properties of Itraconazole capsules showed that they are appropriate to be used for the treatment of infections where plasma levels are not critical. The kinetics of the drug in the skin and nails are different on their own and unique at the same time.

Pharmacokinetics

To study the pharmacokinetics behavior, concentrations of all four stereoisomers were added up because separating the (2R,4S) stereoisomers was not completed. During the in vitro study it was concluded that studying the isomers for their selectivity was more justifiable when done in pairs rather than individually. The plasma concentration versus time curve for the (2R,4S) and (2S,4R) are almost superimposable on each other. The pharmacokinetic studies of itraconazole were crucial to getting a better understanding of why the two pairs of stereoisomers form metabolites while the other pair does not but still show high affinity in regards to inhibition of CYP3A4.

Metabolism

Itraconazole is mainly metabolized in the liver, where it produces over 30 metabolites. The major metabolites, such as hydroxy-itraconazole, have in vitro activity and reach higher plasma concentrations. Itraconazole peak serum levels can be achieved in about 4 to 5 hours after administration of itraconazole OS [Poirier and Cheymol 1998; Stevens 1999]. There is an immediate spike in serum concentrations after i.v infusion of itraconazole [Zhou et al 1998].

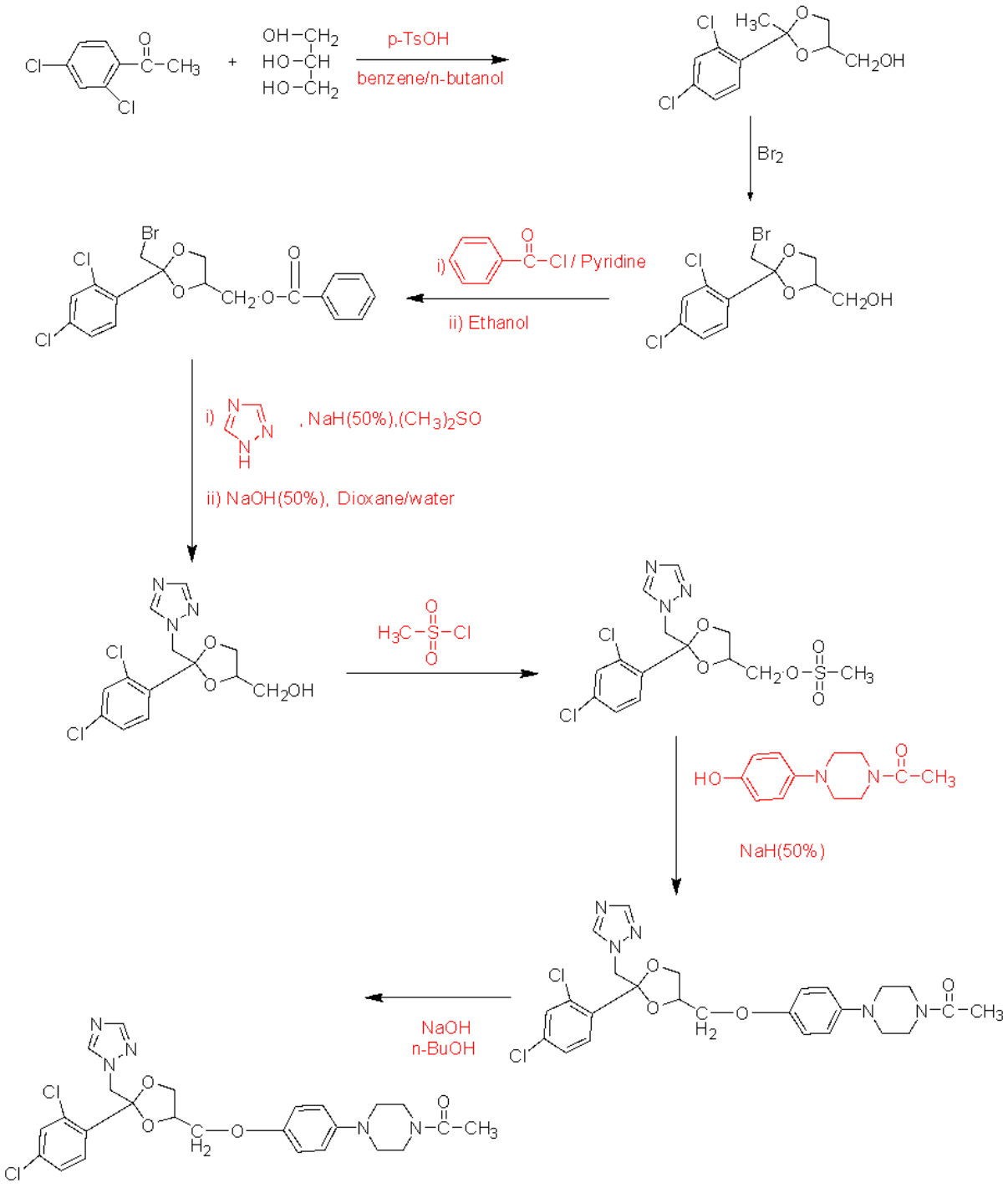
The antifungal property of itraconazole was found to be when it was found to inhibit the P45- and its enzymes. From the triazole nitrogen itraconazole was able to coordinate the heme of the P450 [Bossche et al 1989]. There are a total of eight stereoisomers possible of itraconazole resulting from its three chiral centers. The studies proved that both the *cis* and *trans*-ITZ stereoisomers show great interaction with the CYP3A4. Two out of four cis stereoisomers go through metabolism by CYP34A and a reorientation of the itraconazole within the CYP34A was done to produce metabolite 3'-OH-ITZ [Peng et al 2012]. The *cis* isomers did not show equal metabolizes, (2R,4S,2'S)-ITZ metabolized 5 times the OH-ITZ amount when compared with its counterpart stereoisomer (2R,4S,2'R)-ITZ. The larger amount of production is attributed to a speedier catalytic rate from (2R,4S,2'S)-ITZ. This was one of the three metabolites formed by the itraconazole, the other two were formed after the oxidation of triazole nitrogen was completed resulting in the formation of keto-ITZ, and N-desalkyl-ITZ. The same metabolites were not achieved with the trans isomers. A depletion in the substrate was observed and after analyzing the incubation, two different metabolites were found in the trans isomers. The half-life of the metabolites was found to be shorter than the ITZ compound [Ducharme et al 1995].

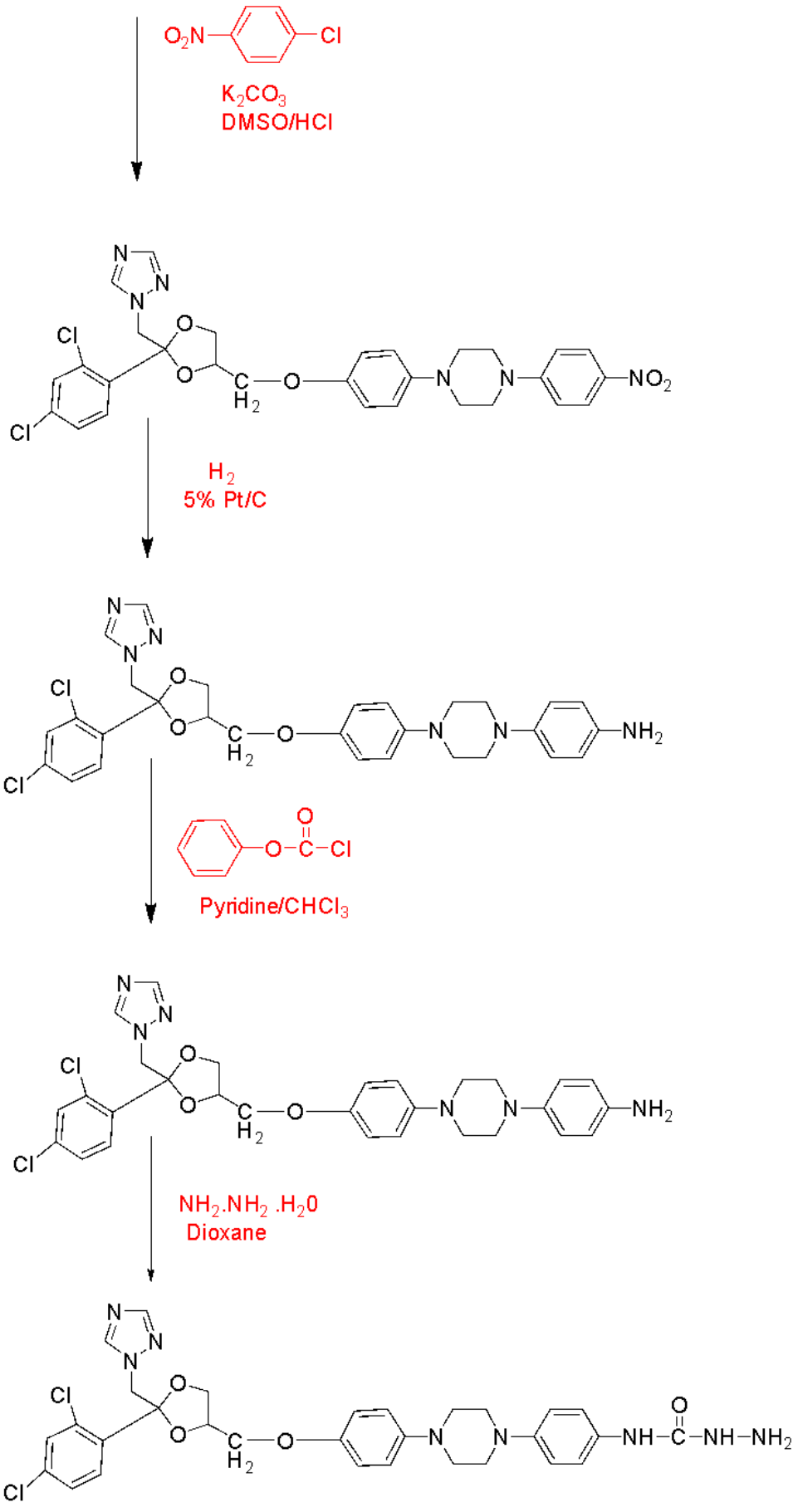
These isomers then underwent trials to understand their properties in showing anti-angiogenic and anti-fungal properties. When compared with the cis isomers counterpart the trans isomers did not show much efficiency in treating angiogenesis. Whereas in the case of antifungal properties two of the trans isomers showed very similar results to the *cis*-diastereomers and were much more efficient in curbing the fungal infections [Shi et al 2010].

CHAPTER 2

SYNTHESIS

The ketalization of 2,4- dichloro acetophenone with glycerine in refluxing benzene was the first step in the production of itraconazole (Scheme 1) [Heeres et al 1979]. The crude product was then brominated at 40 ^{o}C to obtain a 10 in 91 percent yield. Treatment with benzoyl chloride in pyridine preserved the main alcohol as the benzoate, allowing for the isolation of a crystalline solid in 50% yield. The sodium salt of 1,2,4-triazole (produced in situ from triazole and sodium hydride) was treated with the bromide in DMSO at 130^{o}C to provide a variety of regioisomeric triazole derivatives, which were saponified with a mixture of aqueous sodium hydroxide and dioxane [Heeres et al 1983]. Chromatography was used to separate the isomers, with the desired 1-substituted triazole isomer being the main result (about 10: 1). Following mesylation of the alcohol with mesyl chloride in pyridine (87%) and a second nucleophilic displacement with the sodium salt of N-acetyl-4-hydroxyphenylpiperazine at 80^{o}C, which was generated in situ with sodium hydride in DMSO, the triazole analogue of ketoconazole was obtained in 64 percent yield [Heeres 1984]. The final phenyl ring was attached by nucleophilic aromatic substitution using 4-chloronitrobenzene under mildly basic circumstances in DMSO at 120^{o}C after deacetylation with sodium hydroxide in n-butanol under refluxing conditions (70%). The equivalent poorly soluble aniline derivative was obtained by catalytic hydrogenation over platinum on carbon in ethylene glycol monomethyl ether at 50^{o}C, which necessitated heating of the crude reaction mixture to avoid filtration of the product along with the catalyst. The aniline derivative was carbamoylated with phenyl chloroformate in a mixture of chloroform and pyridine to produce an activated carbamate, which was then treated with hydrazine in two steps to obtain the semicarbazide in 86% yield. The cyclized triazolone was obtained by condensing the semicarbazide with formamidine acetate in DMF at 130^{o}C for 3 hours (62% yield). To obtain itraconazole as a crystalline solid from toluene, powdered potassium hydroxide in DMSO was used to alkylate it using 2-bromobutane.


p-TsOH
benzene/n-butanol
Br2
i) Cl / Pyridine
ii) Ethanol
i) , NaH(50%),(CH3)2SO
ii) NaOH(50%), Dioxane/water
NaH(50%)
NaOH
n-BuOH


O2N
Cl
K2CO3
DMSO/HCl
N
N
N
O
O
Cl
Cl
C
H2
O
N
N
NO2
H2
5% Pt/C
NH2
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Pyridine/CHCl3
NH2.NH2 .H20
Dioxane
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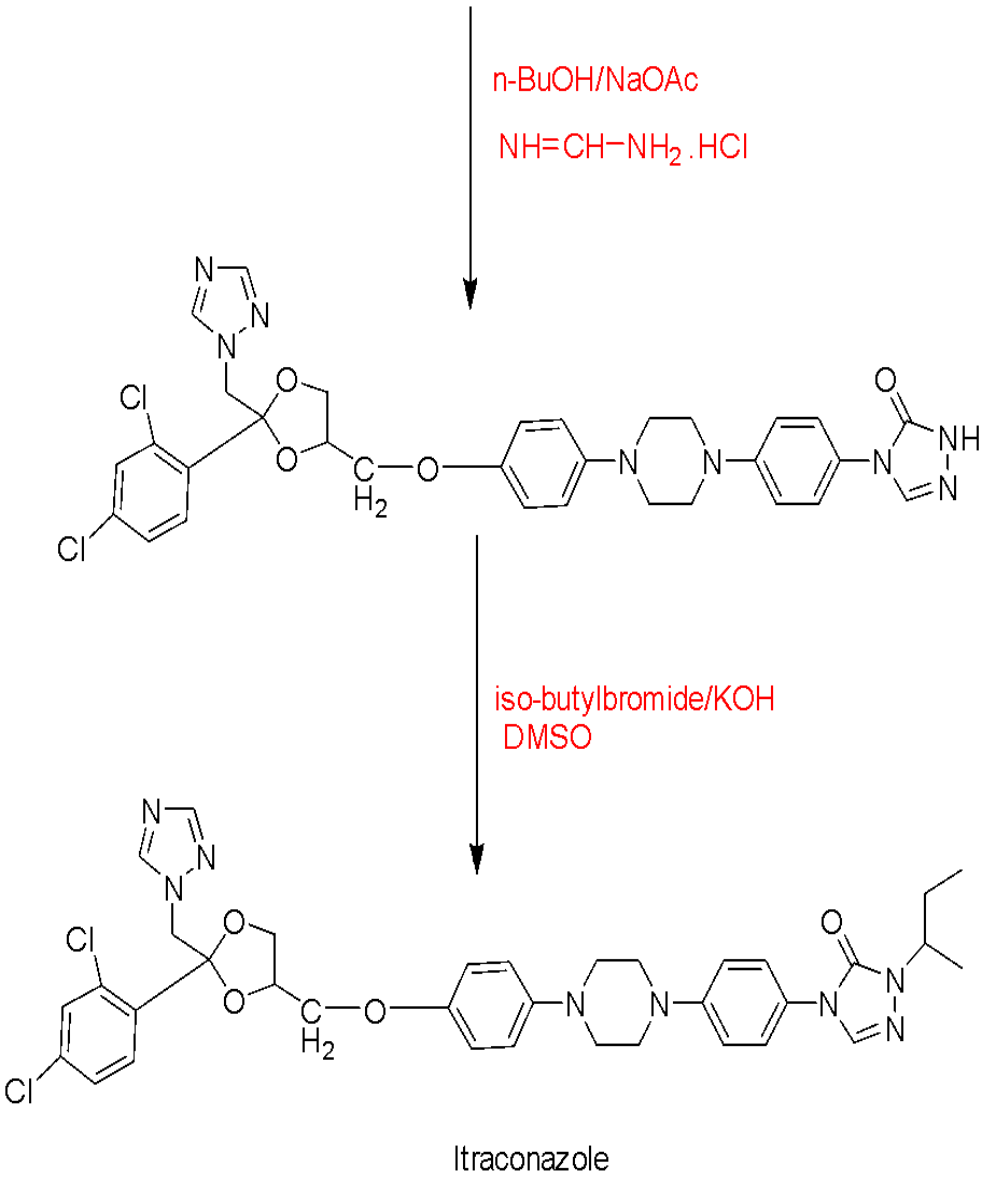
NH
NH2



Itraconazole

scheme 1

CHAPTER 3

ITRACONAZOLE AS ANTIFUNGAL DRUG

3.1. Mechanism of Action

During a fungal infection, lanosterol is converted into ergosterol. To treat the fungal infection itraconazole needs to inhibit the formation of ergosterol. The ergosterol formation is due to the accumulated antecedent intermediate called the 14 alpha-methylsterols [Borgers and Van de Ven 1987; Vanden Bossche 1985]. Demethylation occurs of the 14 alpha-methylsterols which happens due to the activation of cytochrome P-450. The binding of the cytochrome P-450 heme iron with the N-4 nitrogen atom of itraconazole and the hydrophobic part binds to the apoprotein structure of the enzyme [Janssen 1987] inhibits its activation and enzyme function. CYP450 is further divided into 6 enzymes. Here, the CYP3A4 plays a significant role in the drug-drug interaction. The IC50 values of itraconazole for CYP3A4 is 0.0326 µM. The other two enzymes CYP2C9 and CYP2C19 are more than 10 µM [Niwa et al 2005]. The examples of the interactions are listed below. This leads to replication and encourages cell death and in Candida Albicans the yeast cells that are present are subjected to transformation into hypothetically invasive hyphae [Haria et al 1996]. The stereoisomers of ITZ show excellent inhibition of the CYP3A4 by attacking the heme iron. But two stereoisomers of (2R, 4S) bind with heme iron so that the alkyl-side chain is presented to it.

3.2 Inhibition of CYP3A4

The inhibition of ITZ was understood more in-depth with the help of CYP3A4 supersomes. When incubated for two minutes, the itraconazole concentration of (2R, 4S, 2’S)-ITZ and (2R,4S,2’R)-ITZ started to decrease, and the formation of its stereoisomers OH-ITZ and keto-ITZ increased with their respective metabolites, which was generally in the range of 2-44 nM and 1-12 nM respectively. A different set of isomers was incubated for the same duration (2S, 4R, 2’S)-ITZ or (2S, 4R, 2’R)-ITZ but there was no change in their incubations. Despite the fact, that two of the stereoisomers could not undergo metabolism the binding ability of all four stereoisomers with the CYP3A4 was not affected. The triazole nitrogen was able to form bonds with heme iron and stop the inhibition. There was no distinguishable factor that could separate the two (2R, 4S) and two (2S, 4R)-ITZ from each other. A spectrum was characterized for the four stereoisomers, here the (2R, 4S) showed

a slightly higher extinction coefficient with CYP3A4 than the (2S,4R) and all of them were in the range of 434 nm - 390 nm.

3.3 Drug Interactions

Like all the other drugs itraconazole also has the potential to have interactions with other drugs. Mostly because itraconazole is metabolized in the liver by the enzyme CYP3A4. Any other drug being metabolized by the same enzyme allows itraconazole to form bonds with it which may or may not be good for the body. Interaction with some drugs could be life-threatening and caution must be taken while prescribing this medicine [Katz 1999].

CHAPTER 4

ITRACONAZOLE AS ANTIVIRAL DRUG

The need for antiviral drugs in recent years has increased tremendously. After the COVID-19 virus, scientists all around the world are trying to make sure a situation like this doesn't arise again. One of the ways to fight a virus is to build our immunization stronger and secondly, to have proper vaccination systems. But with time even the virus mutates, developing a resistance to our immune system.

Vaccinations or drugs for viral diseases have a very direct approach to the virus, all the while creating resistance towards new mutations. This has already been observed in some viral diseases like the Influenza A virus (IAV) [Hurt et al 2009; Trebbien et al 2014]. In order to tackle this problem, new ways have started to develop to attack the virus. Now instead of targeting the virus directly, the host factors are being targeted that are crucial for the host virus to survive in the body. It prevents the virus from spreading throughout the body as they have the tendency to multiply very quickly. Also, breaking their defense system at the same time. Repurposing drugs that are used for the treatment of other infections is a new approach that has been studied in the past few years.

Viruses may come in different forms but their objective is to attack the body that resides in the host cell which helps in further propagation. In order to emancipate its virions the virus proteins and genome need a transportation mechanism so they attack the basic cellular actions taking place in our body. Once the virus is attached to the host cell, it infiltrates into the cell in the case of a nonenveloped virus, and in an enveloped virus, it follows the fusion process with the plasma membrane. Once penetrated the virus is in full control of the host cell by releasing its genome into it. They then multiply by using the cell organelles of that cell. Virions are released into our body by budding or exocytosis of virion-containing vesicles. Manipulation of cellular signals is done to stop the detection and destruction of the virus and to have a smooth transportation of the virions [Schloer et al 2022].

4.1 Repurposed drugs targeting viral infections

The main objective when targeting the virus is to completely destroy the host cell while subduing the virions spread throughout the body. The repurposed drug can target the virus at any stage of the infection. From the beginning by targeting the host cell interaction with the virus. Preventing the virus proteins and genomes from

spreading to the body and attacking the cell organelles. The repurposed drug can also directly rewire the cellular signaling pathways that were manipulated by the virus. Once the cellular signals are back to their original pathway they can themselves destroy the virus spread in the body [Gordon et al 2020]. Since viruses have a tendency to mutate especially RNA viruses which have a tendency to mutate themselves at a much faster pace [Hameed et al 2020]. One of the key factors that allows the virus to enter into the host cell membrane is an increase in LE/L cholesterol rates. Once the LE/L cholesterol rate spikes up the pH decreases considerably, and the low pH values allow the genome to enter into the cell [Le Blanc et al 2005]. Itraconazole is a great inhibitor of the Niemann-Pick C1 (NPC1) protein, an endolysosomal integral membrane protein. This is required for transporting cholesterol from low-density lipoprotein (LDL) cells [Trinh et al 2017].

4.2 Picornavirus inhibition by Itraconazole

Picornavirus results in the maximum pathogens found in the body. Enterovirus is one of its types which causes diseases like polio. The viral gene is a positive RNA which is imitated by assembling the proteins of virus and its army present in the intracellular membranes known as replication organelles [Strating et al 2015]. In 2010 according to a research done by Hsu NY showed that the replication organelles are designed due to the remolding of virus induced secretory pathway which begin in the Golgi complex. Alterations in the lipid homeostasis due to the virus also plays a key role in making replication organelles [Strating et al 2015].

The crucial involvement of viral proteins 2BC and 3A lies in orchestrating membrane rearrangements by enlisting vital host factors necessary for Enterovirus replication within replication organelles (ROs). These factors include phosphatidylinositol-phosphate-4-kinase III beta (PI4KIIIb), a lipid kinase localized in the Golgi apparatus responsible for producing phosphatidylinositol-4 phosphate (PI4P) [Hsu et al 2010]. According to in vitro analysis done by Hsu it was confirmed that the RNA of a virus binds with PI (4) P, though there was no confirm analysis whether it triggers the infected cells [Hsu et al 2010]. Conferring from Rothwell et al., 2009 and Wang et al., 2014 it’s concluded that many RNA

viruses with positive polarity remolds the cholesterol cells to ensure the genome duplication is executed properly. It was demonstrated that oxysterol-binding protein (OSBP) plays a crucial part in transporting cholesterol and PI4P between the endoplasmic reticulum (ER) and Golgi [Mesmin et al 2013].

4.3 Inhibition of Influenza virus by Itraconazole

Reports of influenza virus being contracted by thousands of people every year in seasonal epidemics pose a great threat to the lives of people. Vaccination is one way to stop the virus from spreading but the current antivirals have not shown good results [Leang et al 2013; Fauci 2006; Lampejo 2020]. When the virus binds to the sialic acid found on the exterior of the cell [Edinger et al 2014] and the early endosomes located near the plasma membrane are converted to late endosomes found near the nucleus the virus transports into the cytoplasm of the cell because of the pH fusion between them [Johannsdottir et al 2009].

The IFN increases cholesterol synthesis in the macrophages and limits its synthesis in the cells. This leads to an increase in type I IFN synthesis and activates the interferon-stimulated genes (ISG) [York et al 2015]. The ISGs may attack all the viruses differently as they are quite selective in nature [Schneider et al 2014]. An increase in IFN synthesis activates the Interferon Trans membrane Protein 3 (IFITM3) which disrupts the pH-dependent fusion between the virion and endosomes. Antiviral activity after the IFITM3 interacted with the cell was inferred due to the amassing of cholesterol in endosomes [Desai et al 2014].

4.4 Itraconazole used in the treatment of COVID-19

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the pandemic that occurred worldwide in 2020. With no vaccination available this pandemic took millions of lives. Medicines already repurposed as antiviral drugs were put to trial to target the virus [Cai et al 2020]. The outer layer of coronavirus is coated with S glycoprotein, responsible for the transmission of the virus in the host cell. The covid 19 virus enters the body by attaching to the angiotensin-converting enzyme 2 (ACE2) cell receptor [Hoffmann et al 2020]. The S protein was then divided into two parts S1 and S2. The S1 through its receptor binding

domain (RBD) enters the host cell by identifying the angiotensin-converting enzyme 2 [Yang et al 2020]. However, since the RBD can easily evolve, it will become difficult to identify the different receptors in different sets of coronavirus. The S2 is responsible for the fusion of the virus into the host cell, the fusion peptide present in the S2 subunit is transmitted into the host cell membrane. The heptapeptide repeat sequence 1 (HR 1) then binds with the heptapeptide repeat sequence 2 (HR2) which leads to the formation of a six-helical bundle (6HB) [Xia et al 2020]. The 6HB helical structure is important for fusion of SARS-CoV-2 into the host cell. There is no interaction between the HR1 and HR2 prior to its fusion while a bridge is formed between the two when the 3-helical bundle of HR1 face-to-face attaches to the HR2. Itraconazole showed great binding affinity to pre-fusion and post-fusion conformers. To get a better understanding of how the virus enters into the cell PsV assays of the SARS-CoV-2 were studied and also to understand how itraconazole inhibited the virus [Xia et al 2020]. Itraconazole has shown great resistance against the SARS-CoV-2 virus as it was able to bind with pre- and post-fusion conformations, thus preventing the formation of the 6HB an essential medium for virus infusion. Also due to its low cell toxicity, it was considered safe to be used in the body. Itraconazole has broad-spectrum antiviral abilities as it was also able to inhibit the SARS-CoV and MERS-CoV through the S protein-mediated route.

CHAPTER 5

ITRACONAZOLE AS ANTICANCER DRUG

Recently, there have been reports suggesting that itraconazole can not only be used for fungal infections but also has the potential antiangiogenic activity both in vitro and in vivo [Chong et al 2007; Aftab et al 2011]. Also, it can inhibit the growth of MB allografts and Hh signaling [Kim et al 2010]. Itraconazole indirectly inhibits mTOR and vascular endothelial growth factor receptor (VEGFR2) functioning and hence attains anti-angiogenic properties [Shi et al 2011].

5.1 Types of mechanisms of action

5.1.1 Inhibition of Antiangiogenic properties

To grasp the structural framework that affects the antiangiogenic activity, a total of eight stereoisomers of the itraconazole molecule were synthesized. There was a distinguishable one pair of trans-stereoisomers activity found after all the stereoisomers were individually evaluated for both their in vitro antiangiogenic and 14 alpha-demethylase inhibition (14DM) [Marichal et al 1990]. As attempts were made to better understand the antiangiogenic activity of itraconazole on a molecular level, it was found that the human umbilical vein endothelial cells (HUVEC) were affected severely both cellular and biochemically [Xu et al 2010].

After a thorough analysis of the activity of previous analogs against HUVEC proliferation, the modifications found on the side chain of the analog molecule did not hinder the activity of itraconazole in any way. In comparison to itraconazole its analogs do not have much potential to restrict the HUVEC proliferation as they generally lack a side chain or in some cases if the side chain is present, they are very short. Exceptional cases like have a considerably higher potency.

A new hypothesis was made when some compounds with a larger side chain were found, also being presumed that the binding site was not sterically hindered. A few other compounds with a rigid conformation also agreed with this hypothesis suggesting that the assumed target may have a deep binding site. However, the loss of activity came as a surprise, hence rejecting this hypothesis. This can be due to the presence of two nitrogen that result in the loss of lipophilicity and further reduce the activity of the compound. The presence of diazirine leads to an increase in lipophilicity. It is later indicated in one of the compounds after its near total loss of

activity that there is a certain limit present to the side chain in retrospect to the inhibition of HUVEC proliferation.

To increase the potential of itraconazole against HUVEC proliferation multiple functional groups were incorporated into the compounds like the azido group, the terminal alkyne, and the cyano group. This indicates that different substituents can replace the sec-butyl part of itraconazole to inspect the itraconazole potential. This conclusion was drawn after a stipulation sighting the fact that the binding site is not completely availed by the sec-butyl group of itraconazole [Shi et al 2011].

5.1.2 Inhibition of Hh pathway

To better understand the inhibition of the Hh pathway by itraconazole let's start the basics with how cancer is developed by the Hh pathway.

Hedgehog activation: The Hh pathway in older tissue cells is active [Shi et al 2011] and it is the over activation of this pathway that ultimately leads to cancer development. PTCH acts as an inhibitor of SMO, but once the Hh pathway is activated, the SMO inhibition by PTCH gets stopped [Xu et al 2010]. This inhibition was the reason that didn’t allow the Hh signals to bind up with the responding cells. Now since the inhibition of SMO is also alleviated, the SMO is shifted to the main cilium present in the cells. This acts as a domino effect of different proteins being activated, mainly the germ line variation of GLI a zinc-finger transcription group. GLI was activated after it was dissociated from the suppressor of fused (SUFU), the SUFU originally acts as a negative regulator of the Hh pathway and also as a suppressor of GLI [Barnfield et al 2005]. SUFU prevented the GLI genes from targeting the cells by regulating the Hh signals and instead bound the GLI to them and harbored them in the cytoplasm. GLI comprises three different parts - GL11, GL12, and GL13. These three members play a key role in cancer development with GL11 and GL12 acting as a trigger factor for cancer cells by enhancing their size and starting a wide spread of cancerous cells in the body [Das et al 2009].

5.1.3 Itraconazole stops tumor progression

Many studies over the years have shown the capability of Itraconazole to prevent cancer from spreading in the body by acting as an inhibitor and blocking the Hh pathway. During one such trial, it was found that itraconazole can be used at a safe concentration, without having any harmful effect on human beings [Kim et al 2010]. For itraconazole to actively stop cancer from spreading throughout the body it needs to block the Hh pathway, SMO, and the GLI proteins to be activated [Liu et al 2019]. From the above paragraph, it was concluded that when the inhibition of SMO was stopped it then led to the activation of GLI, which is the main reason for the spreading of cancer. Multiple studies found that Itraconazole can stop the activation of SMO and GLI, hence, not allowing them to target the cells and slow their mechanisms. Once the inhibition of SMO and GLI is achieved then the Hh pathway itself gets under expressed and stops the growth of many cancers in vivo and in vitro. The target genes that were being attacked by the GLI and SMO were also suppressed and these included the Sox9/mTOR, cyclin D1, Wnt/β-catenin, and Bcl-2/cyt C, these were responsible for the cell cycle arrest and apoptosis. Some other genes like PI3K/AKT/mTOR and VEGFR2 are responsible for targeting autophagy and angiogenesis inhibition.

5.3 Repurposing of Itraconazole for cancer

5.3.1 Itraconazole targeting lung cancer cells

The earliest experiments to understand uses of itraconazole began in 1995 when Asashara gave a study on how the vascular endothelial growth factor (VEGF) and the basic fibroblast growth factor (bFGF) are found to be involved in the angiogenesis affecting tumors. Lung cancer is the mainstream cause of cancer in most patients. The main feature of angiogenesis is endothelial cell migration which is considerably different from its proliferation [Lamalice et al 2007]. Itraconazole successfully prevented the Human umbilical vein endothelial cell (HUVEC) migration by inhibiting the VEGF, bFGF, and EGM-2-triggered migrations [Aftab et al 2011]. Antiangiogenic therapy tends to focus on the antibodies derived that can interact with the endothelial growth factors of the tumor and prevent the ligand

binding with endothelial receptors. Itraconazole inhibits angiogenic signaling pathways by targeting multiple inhibitors which proves it to be more effective. In HUVEC assays the VEGF and bFGF triggered independent growth conditions of the HUVEC proliferation which was steadily inhibited by itraconazole. The inhibition mechanism is cell-type specific. During advanced stages the NSCLC are not curable however they showed great results in the beginning of the drug [Jänne et al 2009]. Observing phosphorylation and RTK suggested that itraconazole can stop the activation of VEGFR2 and FGFR3, furthermore, the changes in these two receptors are not related to other itraconazole inhibitions such as the cholesterol trafficking and mTOR signals.

5.3.2 Inhibition of Breast cancer cells through Itraconazole

Studies carried out by Wang in 2017 giving a complete analysis on how the breast cancer was suppressed after the use of itraconazole. The main study was carried out on the MCF-7 and SKBR-3, two of the breast cancer cell lines were performed to understand the mechanism of attack by the drug [Wang et al 2017]. On the treatment of cancer cells with itraconazole, cell viability decreased leading to an increase in cell death simultaneously preventing the emergence of colonies. The rapidly growing cells in the G0/G1 phase indicated the inhibition of cancer cell development through cell death. Itraconazole significantly increased the caspase-3-activity in the SKBR-3 cells which leads to apoptosis. MCF-7 does not have the caspase-3 expression. Autophagy reduces the growth of cancer cells. An autophagic flux occurred in the cells treated by this drug due to elevated concentrations of autophagic markers called the LC3-II protein. Hh signals are also involved in the cytotoxicity of breast cancer cells.

CHAPTER 6

CONCLUSION

Itraconazole, traditionally an antifungal medication, has shown promise beyond its conventional use, demonstrating significant potential in the treatment of various non-fungal diseases. The repurposing of itraconazole is driven by its multifaceted pharmacological properties, including antiangiogenic, anticancer, and antiviral activities. These diverse effects stem from itraconazole's ability to inhibit hedgehog signaling, angiogenesis, and autophagy, and its impact on cholesterol homeostasis, highlighting its versatile therapeutic potential.

Clinical trials and preclinical studies have revealed itraconazole's efficacy in treating cancers such as basal cell carcinoma, non-small cell lung cancer, and colorectal cancer. Its role in hindering angiogenesis—a critical process in tumor growth and metastasis—underscores its utility as an anticancer agent.

Moreover, itraconazole's antiviral properties have gained attention, especially in the context of emerging viral infections. Its ability to inhibit viral replication positions it as a candidate for treating diseases like influenza and possibly COVID-19. The drug's well-established safety profile and extensive clinical usage history further support its repurposing potential, offering a cost-effective and expedited pathway to novel treatments.

In conclusion, the repurposing of itraconazole represents a promising frontier in medical therapeutics. Its broad-spectrum activity, combined with a robust safety record, makes it an attractive candidate for addressing unmet medical needs across various disease spectrums. Continued research and clinical validation are essential to fully harness its therapeutic potential, paving the way for innovative treatments and improved patient outcomes.

The future scope of itraconazole repurposing is vast and promising, driven by its multifaceted pharmacological properties. Ongoing and future research aims to elucidate its mechanisms further, optimizing its efficacy and safety profiles in non-fungal diseases. The exploration of itraconazole in combination therapies could enhance its therapeutic potential, particularly in oncology, where it may work synergistically with other anticancer agents.

REFERENCES

Aftab, Blake T., Irina Dobromilskaya, Jun O. Liu, and Charles M. Rudin. "Itraconazole inhibits angiogenesis and tumor growth in non–small cell lung cancer." *Cancer research* 71, no. 21 (2011): 6764-6772.

Aftab, Blake T., Irina Dobromilskaya, Jun O. Liu, and Charles M. Rudin. "Itraconazole inhibits angiogenesis and tumor growth in non–small cell lung cancer." *Cancer research* 71, no. 21 (2011): 6764-6772.

Alcántara R, Garibay JM. Itraconazol en dermatomicosis: evaluación multicentrica. Invest. med. int. 1988:281-6.

Almaghrabi, M "Synthesis, Analysis, and Pharmacological Profile of Designer Piperazine Drugs." PhD diss., Auburn University, 2021.

Barnfield, Paul C., Xiaoyun Zhang, Vijitha Thanabalasingham, Minoru Yoshida, and Chi-chung Hui. "Negative regulation of Gli1 and Gli2 activator function by Suppressor of fused through multiple mechanisms." *Differentiation* 73, no. 8 (2005): 397-405.

Borgers, Marcel, and Marie-Anne Van de Ven. "Degenerative changes in fungi after itraconazole treatment." *Reviews of Infectious Diseases* 9, no. Supplement_1 (1987): S33-S42.

Bossche HV, Marichal P, Gorrens J, Coene MC, Willemsens G, Bellens D, Roels I, Moereels H, Janssen PA. Biochemical approaches to selective antifungal activity. Focus on azole antifungals. Mycoses. 1989 Nov;32:35-52.

Cai, Yongfei, Jun Zhang, Tianshu Xiao, Hanqin Peng, Sarah M. Sterling, Richard M. Walsh Jr, Shaun Rawson, Sophia Rits-Volloch, and Bing Chen. "Distinct conformational states of SARS-CoV-2 spike protein." *Science* 369, no. 6511 (2020): 1586-1592.

Cauwenbergh G, Stoffels P. Focus on itraconazole. Indian Journal of Dermatology, Venereology and Leprology. 1991, 57:4.

Chong, Curtis R., Jing Xu, Jun Lu, Shridhar Bhat, David J. Sullivan Jr, and Jun O. Liu. "Inhibition of angiogenesis by the antifungal drug itraconazole." *ACS chemical biology* 2, no. 4 (2007): 263-270.

De Doncker P, Gupta AK, Marynissen G, Stoffels P, Heremans A. Itraconazole pulse therapy for onychomycosis and dermatomycoses: an overview. Journal of the American Academy of Dermatology. 1997 Dec 1;37(6):969-74.

Ducharme MP, Slaughter RL, Warbasse LH, Chandrasekar PH, Van de Velde V, Mannens G, Edwards DJ. Itraconazole and hydroxyitraconazole serum concentrations are reduced more than tenfold by phenytoin. Clinical Pharmacology & Therapeutics. 1995 Dec;58(6):617-24.

Edinger TO, Pohl MO, Stertz S. Entry of influenza A virus: host factors and antiviral targets. J Gen Virol. 2014 Feb;95(Pt 2):263-277.

Fauci AS. Emerging and re-emerging infectious diseases: influenza as a prototype of the host-pathogen balancing act. Cell. 2006 Feb 24;124(4):665-70.

Gordon, David E., Gwendolyn M. Jang, Mehdi Bouhaddou, Jiewei Xu, Kirsten Obernier, Kris M. White, Matthew J. O'Meara et al. "A SARS-CoV-2 protein interaction map reveals targets for drug repurposing." *Nature* 583, no. 7816 (2020): 459-468.

Grant SM, Clissold SP. Itraconazole: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in superficial and systemic mycoses. Drugs. 1989, 37:310-44.

Hameed, Raghdah Maytham, Mohanad Kadhim Mirdan Al-Ibraheemi, Falah Hasan Obayes Al-Khikani, Noor Flayyih Hasan, Huda Ali Salman Almosawey, and Atyaf Ali Al-Asadi. "The possible role of immunoglobulin A monoclonal antibodies against COVID-19 infection." *Matrix Science Medica* 4, no. 4 (2020): 96-102.

Haria, Malini, Harriet M. Bryson, and Karen L. Goa. "Itraconazole: a reappraisal of its pharmacological properties and therapeutic use in the management of superficial fungal infections." *Drugs* 51 (1996): 585-620.

Heeres, J. "Structure-activity relationships in a group of azoles, with special reference to 1, 3-dioxolan-2-ylmethyl derivatives." *Pesticide science* 15, no. 3 (1984): 268-279.

Heeres, J., L. J. J. Backx, J. H. Mostmans, and J. Van Cutsem. "Antimycotic imidazoles. Part 4. Synthesis and antifungal activity of ketoconazole, a new potent orally active broad-spectrum antifungal agent." *Journal of medicinal chemistry* 22, no. 8 (1979): 1003-1005.

Heeres, J., R. Hendrickx, and J. Van Cutsem. "Antimycotic azoles. 6. Synthesis and antifungal properties of terconazole, a novel triazole ketal." *Journal of Medicinal Chemistry* 26, no. 4 (1983): 611-613.

Heykants J, Van Peer A, Van de Velde V, Van Rooy P, Meuldermans W, Lavrijsen K, Woestenborghs R, Van Cutsem J, Cauwenbergh G. The clinical pharmacokinetics of itraconazole: an overview. mycoses. 1989, 32:67-87.

Hoffmann, Markus, Hannah Kleine-Weber, Simon Schroeder, Nadine Krüger, Tanja Herrler, Sandra Erichsen, Tobias S. Schiergens et al. "SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor." *cell* 181, no. 2 (2020): 271-280.

Hsu, Nai-Yun, Olha Ilnytska, Georgiy Belov, Marianita Santiana, Ying-Han Chen, Peter M. Takvorian, Cyrilla Pau et al. "Viral reorganization of the secretory pathway generates distinct organelles for RNA replication." *Cell* 141, no. 5 (2010): 799-811.

Hurt, Aeron C., Jessica K. Holien, Michael W. Parker, and Ian G. Barr. "Oseltamivir resistance and the H274Y neuraminidase mutation in seasonal, pandemic, and highly pathogenic influenza viruses." *Drugs* 69 (2009): 2523-2531.

Jänne, Pasi A., Nathanael Gray, and Jeff Settleman. "Factors underlying sensitivity of cancers to small-molecule kinase inhibitors." *Nature reviews Drug discovery* 8, no. 9 (2009): 709-723.

Janssen, P. A. J. "New antifungal agents: a result of target-oriented drug design." In *Frontiers in Microbiology: From Antibiotics to AIDS*, pp. 29-46. Dordrecht: Springer Netherlands, 1987.

Katz, H. I. "Drug interactions of the newer oral antifungal agents." *British Journal of Dermatology* 141, no. s56 (1999): 26-32.

Kim, James, Jean Y. Tang, Ruoyu Gong, Jynho Kim, John J. Lee, Karl V. Clemons, Curtis R. Chong et al. "Itraconazole, a commonly used antifungal that inhibits Hedgehog pathway activity and cancer growth." *Cancer cell* 17, no. 4 (2010): 388-399.

Kim, James, Jean Y. Tang, Ruoyu Gong, Jynho Kim, John J. Lee, Karl V. Clemons, Curtis R. Chong et al. "Itraconazole, a commonly used antifungal that inhibits

Hedgehog pathway activity and cancer growth." *Cancer cell* 17, no. 4 (2010): 388-399.

Lamalice, Laurent, Fabrice Le Boeuf, and Jacques Huot. "Endothelial cell migration during angiogenesis." *Circulation research* 100, no. 6 (2007): 782-794.

Lampejo T. Influenza and antiviral resistance: an overview. Eur J Clin Microbiol Infect Dis. 2020 Jul;39(7):1201-1208. doi: 10.1007/s10096-020-03840-9. Epub 2020 Feb 13.

Larosa E, Cauwenbergh G, Cilli P, Woestenborghs R, Heykants J. Itraconazole pharmacokinetics in the female genital tract: plasma and tissue levels in patients undergoing hysterectomy after a single dose of 200 mg itraconazole. European Journal of Obstetrics & Gynecology and Reproductive Biology. 1986 Oct 1;23(1-2):85-9.

Le Blanc, Isabelle, Pierre-Philippe Luyet, Véronique Pons, Charles Ferguson, Neil Emans, Anne Petiot, Nathalie Mayran et al. "Endosome-to-cytosol transport of viral nucleocapsids." *Nature cell biology* 7, no. 7 (2005): 653-664.

Leang SK, Deng YM, Shaw R, Caldwell N, Iannello P, Komadina N, Buchy P, Chittaganpitch M, Dwyer DE, Fagan P, Gourinat AC, Hammill F, Horwood PF, Huang QS, Ip PK, Jennings L, Kesson A, Kok T, Kool JL, Levy A, Lin C, Lindsay K, Osman O, Papadakis G, Rahnamal F, Rawlinson W, Redden C, Ridgway J, Sam IC, Svobodova S, Tandoc A, Wickramasinghe G, Williamson J, Wilson N, Yusof MA, Kelso A, Barr IG, Hurt AC. Influenza antiviral resistance in the Asia-Pacific region during 2011. Antiviral Res. 2013 Feb;97(2):206-10.

Liu, Musang, Guanzhao Liang, Hailin Zheng, Nan Zheng, Hu Ge, and Weida Liu. "Triazoles bind the C-terminal domain of SMO: Illustration by docking and molecular dynamics simulations the binding between SMO and triazoles." *Life sciences* 217 (2019): 222-228.

Marichal, P., J. Gorrens, and M. C. Coene. "Biochemical basis for the activity and selectivity of oral antifungal drugs." *British journal of clinical practice. Supplement* 71 (1990): 41-46.

Matthieu L, De Doncker P, Cauwenbergh G, Woestenborghs R, Van de Velde V, Janssen PA, Dockx P. Itraconazole penetrates the nail via the nail matrix and the

nail bed—an investigation in onychomycosis. Clinical and experimental dermatology. 1991 Sep 1;16(5):374-6.

Mesmin, Bruno, Joëlle Bigay, Joachim Moser Von Filseck, Sandra Lacas-Gervais, Guillaume Drin, and Bruno Antonny. "A four-step cycle driven by PI (4) P hydrolysis directs sterol/PI (4) P exchange by the ER-Golgi tether OSBP." *Cell* 155, no. 4 (2013): 830-843.

Negroni R, Arechavala AI. Itraconazole: pharmacokinetics and indications. Archives of medical research. 1993, 24(4):387-93.

Niwa, Toshiro, Toshifumi Shiraga, and Akira Takagi. "Effect of antifungal drugs on cytochrome P450 (CYP) 2C9, CYP2C19, and CYP3A4 activities in human liver microsomes." *Biological and Pharmaceutical Bulletin* 28, no. 9 (2005): 1805-1808.

Peng CC, Shi W, Lutz JD, Kunze KL, Liu JO, Nelson WL, Isoherranen N. Stereospecific metabolism of itraconazole by CYP3A4: dioxolane ring scission of azole antifungals. Drug metabolism and disposition. 2012 Mar 1;40(3):426-35.

Piérard GE, Arrese JE, Piérard-Franchimont C. Itraconazole. Expert Opinion on Pharmacotherapy. 2000, 1(2):287-304.

Poirier JM, Cheymol G. Optimisation of itraconazole therapy using target drug concentrations. Clinical pharmacokinetics. 1998 Dec;35:461-73.

Prentice AG, Glasmacher A. Making sense of itraconazole pharmacokinetics. Journal of Antimicrobial Chemotherapy. 2005 Sep 1;56(suppl_1):i17-22.

Schloer, Sebastian, Jonas Goretzko, and Ursula Rescher. "Repurposing antifungals for host-directed antiviral therapy?." *Pharmaceuticals* 15, no. 2 (2022): 212.

Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. Annu Rev Immunol. 2014;32:513-45.

Shi W, Nacev BA, Bhat S, Liu JO. Impact of absolute stereochemistry on the antiangiogenic and antifungal activities of itraconazole. ACS medicinal chemistry letters. 2010 Jul 8;1(4):155-9.

Shi, Wei, Benjamin A. Nacev, Blake T. Aftab, Sarah Head, Charles M. Rudin, and Jun O. Liu. "Itraconazole side chain analogues: structure–activity relationship studies for inhibition of endothelial cell proliferation, vascular endothelial growth factor receptor 2 (VEGFR2) glycosylation, and Hedgehog signaling." *Journal of medicinal chemistry* 54, no. 20 (2011): 7363-7374

Stevens DA. Itraconazole in cyclodextrin solution. Pharmacotherapy. 1999 May;19(5):603-11. doi: 10.1592/phco.19.8.603.31529. PMID: 10331823.

Strating, Jeroen RPM, Lonneke van der Linden, Lucian Albulescu, Joelle Bigay, Minetaro Arita, Leen Delang, Pieter Leyssen et al. "Itraconazole inhibits enterovirus replication by targeting the oxysterol-binding protein." *Cell reports* 10, no. 4 (2015): 600-615.

Trebbien, Ramona, Svend Stenvang Pedersen, Kristine Vorborg, Kristina Træholt Franck, and Thea Kølsen Fischer. "Development of oseltamivir and zanamivir resistance in influenza A (H1N1) pdm09 virus, Denmark, 2014." *Eurosurveillance* 22, no. 3 (2017): 30445.

Trinh, Michael N., Feiran Lu, Xiaochun Li, Akash Das, Qiren Liang, Jef K. De Brabander, Michael S. Brown, and Joseph L. Goldstein. "Triazoles inhibit cholesterol export from lysosomes by binding to NPC1." *Proceedings of the National Academy of Sciences* 114, no. 1 (2017): 89-94.

Van Cauteren H, Heykants J, De Coster R, Cauwenbergh G. Itraconazole: pharmacologic studies in animals and humans. Reviews of infectious diseases. 1987, 9(Suppl_1):S43-6.

Van Cutsem, J. F. M. M. P., F. Van Gerven, M. A. Van de Ven, M. Borgers, and P. A. Janssen. "Itraconazole, a new triazole that is orally active in aspergillosis." *Antimicrobial agents and chemotherapy* 26, no. 4 (1984): 527-534.

Vanden Bossche H, Bellens D, Cools W, Gorrens J, Marichal P, Verhoeven H, Willemsens G, De Coster R, Beerens D, Haelterman C, Coene MC. Cytochrome p-450: Target for itraconazole. Drug Development Research. 1986 May;8(1-4):287-98.

Vanden Bossche, H. "Biochemical targets for antifungal azole derivatives: hypothesis on the mode of action." *Current topics in medical mycology* (1985): 313-351.

Wang, Xiaoya, Sanhua Wei, Yong Zhao, Changhong Shi, Peijuan Liu, Caiqin Zhang, Yingfeng Lei, et al. "Anti-proliferation of breast cancer cells with itraconazole: Hedgehog pathway inhibition induces apoptosis and autophagic cell death." *Cancer Letters* 385 (2017): 128-136.

Westerberg DP, Voyack MJ. Onychomycosis: current trends in diagnosis and treatment. American family physician. 2013, 88(11):762-70.

Willems L, Van der Geest R, De Beule K. Itraconazole oral solution and intravenous formulations: a review of pharmacokinetics and pharmacodynamics. Journal of Clinical Pharmacy and Therapeutics. 2001, 26(3):159-69.

Willems L, Van der Geest R, De Beule K. Itraconazole oral solution and intravenous formulations: a review of pharmacokinetics and pharmacodynamics. Journal of clinical pharmacy and therapeutics. 2001, 26(3):159-69.

Wishart JM. The influence of food on the pharmacokinetics of itraconazole in patients with superficial fungal infection. Journal of the American Academy of Dermatology. 1987 Aug 1;17(2):220-3.

Xia, Shuai, Yun Zhu, Meiqin Liu, Qiaoshuai Lan, Wei Xu, Yanling Wu, Tianlei Ying et al. "Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein." *Cellular & molecular immunology* 17, no. 7 (2020): 765-767.

Xu, Jing, Yongjun Dang, Yunzhao R. Ren, and Jun O. Liu. "Cholesterol trafficking is required for mTOR activation in endothelial cells." *Proceedings of the National Academy of Sciences* 107, no. 10 (2010): 4764-4769.

Yang, Ren, Jiaming Lan, Baoying Huang, A. Ruhan, Mingqing Lu, Wen Wang, Wenling Wang et al. "Lack of antibody-mediated cross-protection between SARS-CoV-2 and SARS-CoV infections." *EBioMedicine* 58 (2020).

York AG, Williams KJ, Argus JP, Zhou QD, Brar G, Vergnes L, Gray EE, Zhen A, Wu NC, Yamada DH, Cunningham CR, Tarling EJ, Wilks MQ, Casero D, Gray DH, Yu AK, Wang ES, Brooks DG, Sun R, Kitchen SG, Wu TT, Reue K, Stetson DB, Bensinger SJ. Limiting Cholesterol Biosynthetic Flux Spontaneously Engages Type I IFN Signaling. Cell. 2015 Dec 17;163(7):1716-29.

Zhou H, Goldman M, Wu J, Woestenborghs R, Hassell AE, Lee P, Baruch A, Pesco-Koplowitz L, Borum J, Wheat LJ. A pharmacokinetic study of intravenous itraconazole followed by oral administration of itraconazole capsules in patients with advanced human immunodeficiency virus infection. J Clin Pharmacol. 1998 Jul;38(7):593-602. doi: 10.1002/j.1552-4604.1998.tb04465.x. PMID: 9702843.